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The genus *Pythium* in Taiwan (2) — an Illustrated diagnostic key

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Abstract – A diagnostic key has been developed to facilitate the identification of 48 species of *Pythium* recorded in Taiwan so far. Photomicrographs are provided for the 10 most common or economically important species to highlight their major morphological characteristics.

Key words – *Pythiaceae*, oomycetes, *Chromista*, *Straminopila*

Introduction

In a review of the genus *Pythium* in Taiwan, Ho (2009) reported that a total of 48 *Pythium* species have been recorded. This is the second part of the paper, presenting a diagnostic key to all these species. Although taxonomic keys to Chinese *Pythium* species are already available (Yu & Ma 1989, Yu 1998), a similar key is lacking for Taiwan. Considering the importance of the genus in the ecosystem (Plaats-Niterink 1981), plant pathology (Hendrix & Campbell 1973), fish diseases (Khulbe 2009), the potential benefits to human beings as agents biological controls of soil-borne diseases (Jones 1995) and mosquitoes (Su et al. 2001), and as a source of enzymes useful in medicine, food industry and the production of biofuel (Stredansky et al. 2000, Mendez-Vilas 2009), there is a need for a diagnostic key to all species of *Pythium* in Taiwan to assist mycologists, plant pathologists, and other scientists, which is presented below. Photomicrographs of the ten most common or economically important species are also provided to facilitate identification.

Materials & methods

Morphological characters used traditionally to classify *Pythium* species are limited and often variable and overlapping. These include the characteristics of the oogonia, oospores, antheridia, sporangia, and hyphae as well as maximum growth temperatures. Moreover, there has been controversy and confusion regarding the terminology of some important taxonomic criteria.

For instance, traditionally, an oospore is considered plerotic if it fills up or nearly fills up the space of the oogonium that produces it (Middleton 1943, Waterhouse 1967,

Plaats-Niterink 1981). More recently, Dick (1990) devised a mathematical formula to calculate the aplerotic index showing the percentage of the oogonial volume occupied by the oospore where an oospore is considered plerotic when the aplerotic index exceeds 70% and aplerotic when the aplerotic index is less than 60%. I have opted for the traditional concept in using the terms plerotic and aplerotic in this key.

There are some species that form sporangia-like structures but no zoospores. These structures have been termed "sporangia" (Middleton 1943), "hyphal bodies" (Waterhouse 1967, Dick 1990), or "hyphal swellings" (Plaats-Niterink 1981). In this key, "zoosporangia" refers to sporangia that produce zoospores and "hyphal swellings" refer to sporangia-like swellings producing no zoospores.

A diagnostic key to the species of *Pythium* in Taiwan

1a. Oogonia produced in single cultures **2**
1b. Oogonia not or rarely produced in single cultures **27**

2a.(1a). Oogonial wall ornamented with projections **3**
2b. Oogonial wall smooth **10**

3a.(2a). Projections numerous, evenly distributed **4**
3b. Projections 0–5 per oogonium, unevenly distributed . . . . . ***P. irregulare*** Buisman

4a.(3a). Zoosporangia absent **5**
4b. Zoosporangia present **6**

5a.(4a). Projections digitate . . . . . ***P. spinosum*** Sawada **var. *spinosum*** (Fig. 1)
5b. Projections conical, acute . . . . . ***P. hydnosporum*** (Mont.) J. Schröt.

6a.(4a). Zoosporangia proliferating internally; chlamydospores large, av. 50 µm diam . . . . . ***P. dimorphum*** F.F. Hendrix & W.A. Campb.
6b. Zoosporangia non-proliferating; chlamydospores absent **7**

7a.(6b). Projections conical, acute; antheridia often absent . . . ***P. oligandrum*** Drechsler
7b. Projections conical, sub-acute; antheridia present **8**
7c. Projections conical, obtuse; zoosporangia lobulate . . . . . ***P. periplocum*** Drechsler
7d. Projections conical, obtuse or mammiform; zoosporangia not lobulate **9**

8a.(7b). Projections mostly 2–3 µm long; zoosporangia often forming discrete complexes of subglobose and inflated elements . . . . . ***P. acanthicum*** Drechsler (Fig. 2)
8b. Projections mostly 3–5 µm long; zoosporangia ellipsoidal to elongated . . . . . ***P. helicandrum*** Drechsler

9a.(7d). Projections mostly 2–6 µm long; zoosporangia globose to ovoid . . . . . ***P. mamillatum*** Meurs.
9b. Projections mostly 5–8 µm long; zoosporangia subglobose to broadly ovoid (oogonia large, av. 53 µm diam) . . . . . ***P. polymastum*** Drechsler

10a.(2b). Zoosporangia filamentous **11**
10b. Zoosporangia lobulate **15**
10c. Zoosporangia globose to ovoid **20**
10d. Zoosporangia absent (hyphal bodies lobulate) . . . . . ***P. tardicrescens*** Vanterp.

11a.(10a). Oogonia <15 μm diam ..... ***P. sukuiense*** W.H. Ko, S.Y. Wang & P.J. Ann
11b. Oogonia >15 μm diam **12**

12a.(11b). Oospore wall pale lilac in color ..... ***P. coloratum*** Vaartja
12b. Oospore wall uncolored or not pale lilac in color **13**

13a.(12b). Oospores plerotic; antheridia stalked ..... ***P. monospermum*** Pringsh.
13b. Oospores aplerotic; antheridia stalked **14**
13c. Oospores aplerotic to nearly plerotic; antheridia with short stalks or sessile
..... ***P. dissotocum*** Drechsler (Fig. 3)

14a.(13b). Antheridia often several from the same stalk, 1–4 antheridia per oogonium, terminal or intercalary ..... ***P. adhaerens*** Sparrow
14b. Antheridia usually one per stalk; 1(–2) per oogonium, mostly terminal ..... ***P. gracile*** Schenk

15a.(10b). Antheridia often intercalary **16**
15b. Antheridia usually not intercalary **17**

16a.(15a). Oogonial stalks straight ..... ***P. aphanidermatum*** (Edson) Fitzp. (Fig. 4)
16b. Oogonial stalks mostly curved towards the antheridia ***P. deliense*** Meurs. (Fig. 5)

17a.(15b). Oospores plerotic **18**
17b. Oospores aplerotic **19**

18a.(17a). Antheridia mostly diclinous; 15–20 antheridia per oogonium
..... ***P. arrhenomanes*** Drechsler
18b. Antheridia mostly monoclinous, 1–2 antheridia per oogonium
..... ***P. torulosum*** Coker & P. Patt.
18c. Antheridia mostly monoclinous; 1–6 antheridia per oogonium
..... ***P. graminicola*** Subraman.

19a.(17b). Maximum growth temperature >40°C ... ***P. myriotylum*** Drechsler (Fig. 6)
19b. Maximum growth temperature <40°C; antheridial branch entwining oogonial stalk ..... ***P. volutum*** Vanterp. & Truscott
19c. Maximum growth temperature <40°C; antheridial branch not entwining oogonial stalk ..... ***P. aristosporum*** Vanterp.

20a.(10c). Zoosporangia non-proliferating or occasionally proliferating internally; oogonia <30 μm diam **21**
20b. Zoosporangia proliferating internally; oogonia >30 μm diam **26**
20c. Zoosporangia absent; oogonia <30 μm diam .....
..... ***P. ultimum*** Trow **var. *ultimum*** (Fig. 7)

21a.(20a). Oospores plerotic **22**
21b. Oospores aplerotic **23**

22a.(21a). Antheridia hypogynous or monoclinous, oogonia often in chain
..... ***P. rostratum*** E.J. Butler
22b. Antheridia absent or monoclinous; oogonia often in chain
..... ***P. salpingophorum*** Drechsler
22c. Antheridia diclinous or monoclinous; oogonia not in chain .. ***P. pleroticum*** T. Itô

23a.(21b). Antheridia bell-shaped **24**
23b. Antheridia not bell-shaped **25**

24a.(23a). Sporangia globose, pyriform or ovoid, occasionally proliferating internally; irregularly elongated antheridia also present ..... ***P. vexans*** de Bary (Fig. 8)
24b. Sporangia various shaped, non-proliferating; elongated antheridia not present ..... ***P. perplexum*** H. Kouyeas & Theoh.

25a.(23b). Antheridia mostly hypogynous; sporangia and/or oogonia mostly intercalary and occasionally catenulate ..... ***P. pulchrum*** Minden
25b. Antheridia variable, mostly monoclinous, sessile but occasionally hypogynous; oogonia often intercalary or catenulate; sporangia terminal or intercalary ..... ***P. paroecandrum*** Drechsler
25c. Antheridia mostly monoclinous, stalked; oogonia terminal and occasionally intercalary; sporangia terminal or intercalary ..... ***P. debaryanum*** R. Hesse

26a.(20b). Antheridia smooth in contour; oogonia terminal; maximum growth temperature >40°C ..... ***P. helicoides*** Drechsler (Fig. 9)
26b. Antheridia often wavy in contour; oogonia terminal; maximum growth temperature <40°C ..... ***P. oedichilum*** Drechsler
26c. Antheridia often wavy in contour; oogonia intercalary or lateral ..... ***P. palingenes*** Drechsler

27a.(1b). Zoosporangia non-proliferating, non-papillate **28**
27b. Zoosporangia proliferating internally, papillate **31**
27c. Zoosporangia absent **33**

28a. (27a). Zoosporangia filamentous ..... ***P. afertile*** Kanouse & T. Humphrey
28b. Zoosporangia spherical, ovoid to cylindrical **29**
28c. Zoosporangia lobulate **30**

29a.(28b). Zoosporangia terminal, spherical, catenulate in water, deciduous, zoospores rare ..... ***P. intermedium*** de Bary
29b. Zoosporangia terminal or intercalary, spherical, pyriform to cylindrical; exit tube very long, up to 150 µm ..... ***P. elongatum*** V.D. Matthews

30a.(28c). Spherical hyphal swellings (10–20 µm) catenulate in water, not deciduous ..... ***P. catenulatum*** V.D. Matthews
30b. Toruloid hyphal elements present; zoospores rare .... ***P. inflatum*** V.D. Matthews

31a.(27b). Zoosporangia ellipsoidal, up to more than100 µm long, terminal on sporangiophore ..... ***P. undulatum*** H.E. Petersen
31b. Zoosporangia globose, pyriform to asymmetrically utriform, often transversely attached to sporangiophore ..... ***P. marsipium*** Drechsler
31c. Zoosporangia globose to ovoid, terminal on sporangiophore **32**

32a.(31c). Zoosporangia av. 25 µm diam; hyphal swellings catenulate ..... ***P. carolinianum*** V.D. Matthews
32b. Zoosporangia av. 30 µm diam ..... ***P. middletonii*** Sparrow

33a.(27c). Hyphal swellings large, globose, mostly 30–40(–49) µm diam and terminal ..... ***P. splendens*** Hans Braun (Fig. 10)
33b. Hyphal swellings globose to ovoid, mostly 25–30 µm diam, intercalary or terminal ..... ***P. sylvaticum*** W.A. Campb. & F.F. Hendrix

Discussion

I have not attempted to address the difficult problem of speciation in the genus *Pythium* in the above key and all species recorded in Taiwan have been accepted without prejudice. Although Plaats-Niterink (1981) considered *P. debaryanum* to be conspecific with *P. ultimum* var. *ultimum*, and *P. gracile* with *P. monospermum*, I have accepted the thesis of Waterhouse (1976) and Dick (1990) that they are recognizably distinct taxa. Similarly, *P. afertile* is retained in this key, as in the keys developed by Waterhouse (1967) and Dick (1990), despite the fact that it was rejected by Plaats-Niterink (1981). The preparation of a traditional diagnostic key for all species of *Pythium* in Taiwan is only the first step in the study of the taxonomy of the genus on the island. Hopefully, in the future, with a combination of morphological studies and the utilization of modern molecular biology techniques, a clearer picture of the speciation of *Pythium* in Taiwan will emerge.

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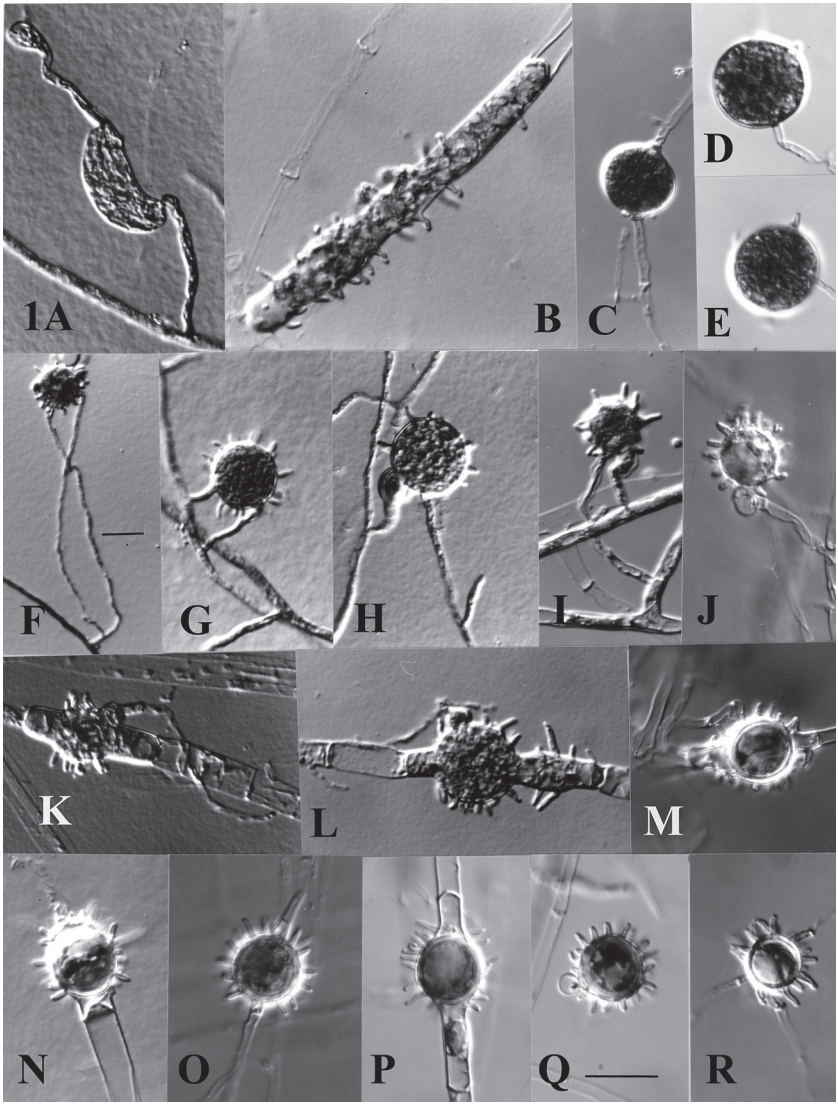

Fig. 1. *Pythium spinosum* var. *spinosum*. **A.** appressorium; **B.** digitate appendages on an intercalary hyphal swelling; **C.** intercalary hyphal swelling; **D, E.** terminal hyphal swellings; **F–J.** ornamented terminal oogonia with stalked antheridia, monoclinous in F, G, and diclinous in H–J; **K–P.** ornamented intercalary oogonia with antheridia stalked and monoclinous in K–M but hypogynous in N–P; **Q, R.** oogonia with plerotic oospores. Scale bar: 20 μm (all at the same magnification)

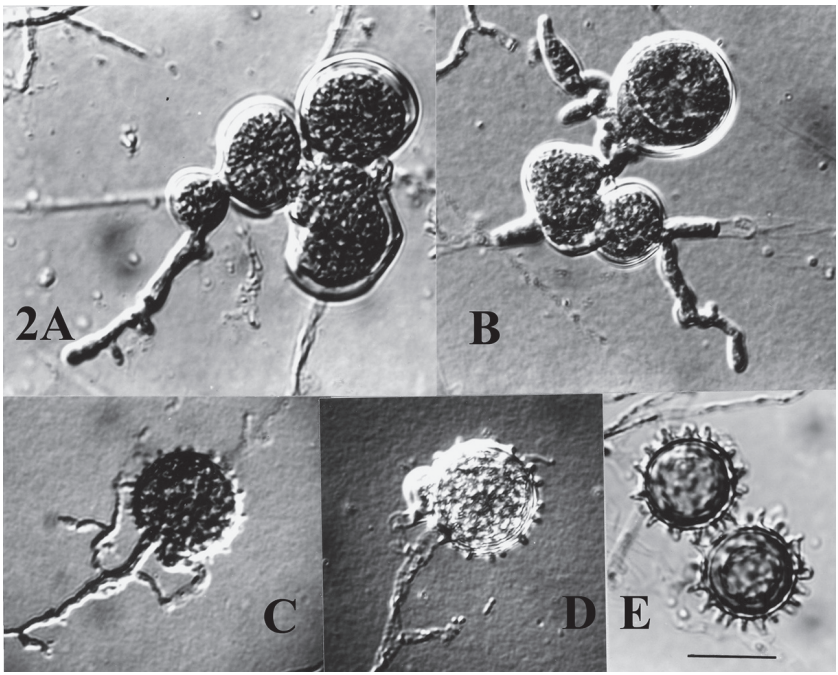

Fig. 2. *Pythium acanthicum*. **A, B.** subglobose sporangial complex; **C, D.** terminal oogonia with monoclinous antheridia; oogonia with short, subacute conical projections and plerotic oospores. Scale bar: 20 μm (all at the same magnification)

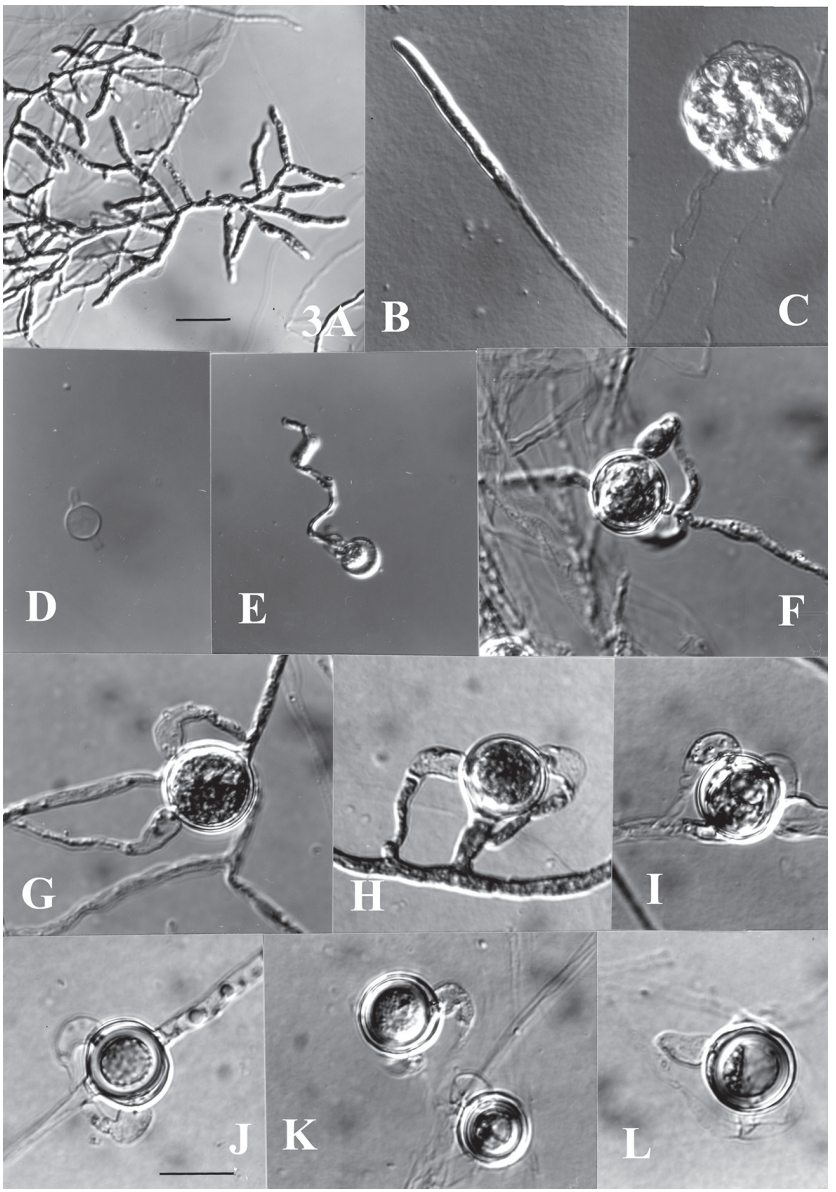

Fig. 3. *Pythium dissotocum*. **A, B.** hyphal tip forming filamentous sporangium; **C.** zoospores in a vesicle at the tip of an exit tube; **D.** Empty cyst with an exit tube after the cyst has germinated by repeated emergence of zoospore; **E.** Cyst germinates by a germ tube; **F–J.** oogonia with short stalked monoclinous antheridia; **K, L.** oogonia with aplerotic to nearly plerotic oospores Scale bar: 20 μm (all at the same magnification except A)

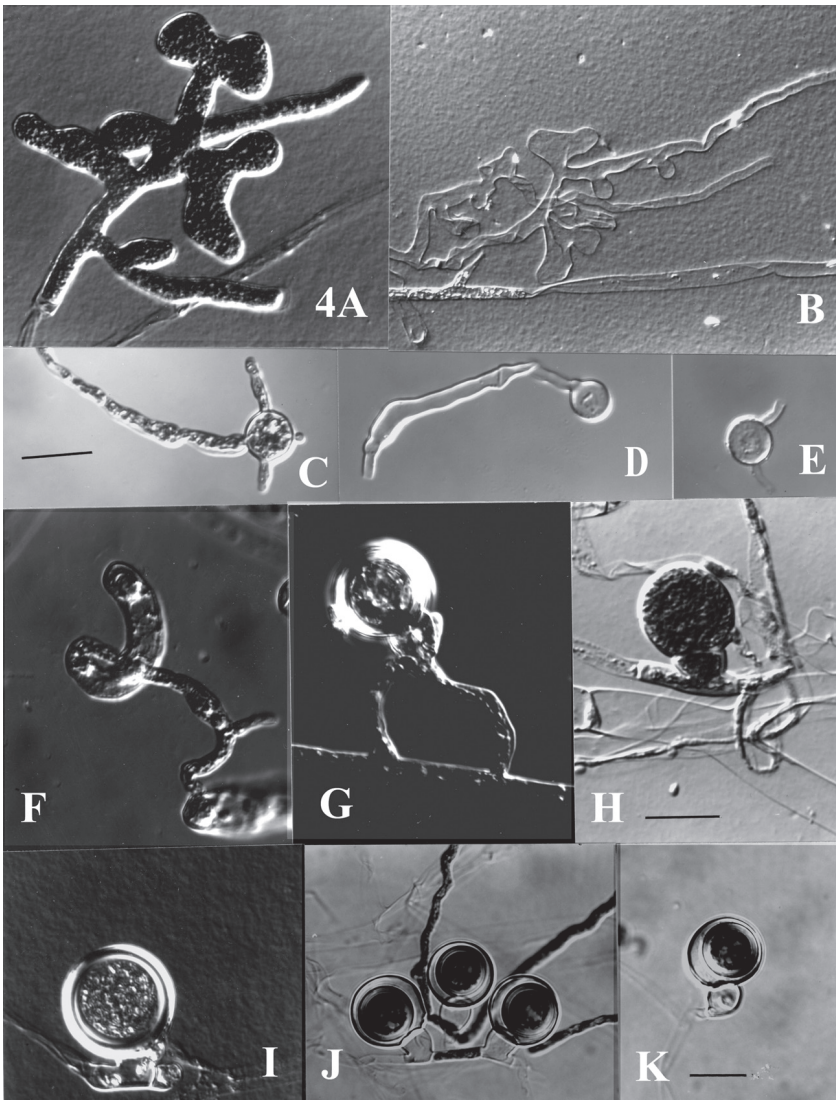

Fig. 4. *Pythium aphanidermatum.* **A, B.** lobulate sporangia, sporangium empty with long exit tube in B after zoospore release; **C–D.** cyst germination by germ tube(s); **E.** Empty cyst with two exit tubes after germination by repeated emergence of zoospore; **F.** appressorium; **G.** oogonium with a terminal, stalked monoclinous antheridium; **H–J.** oogonia with intercalary antheridia; **K.** oospore with aplerotic oospore. Scale bar: 20 μm (all at the same magnification except C and K)

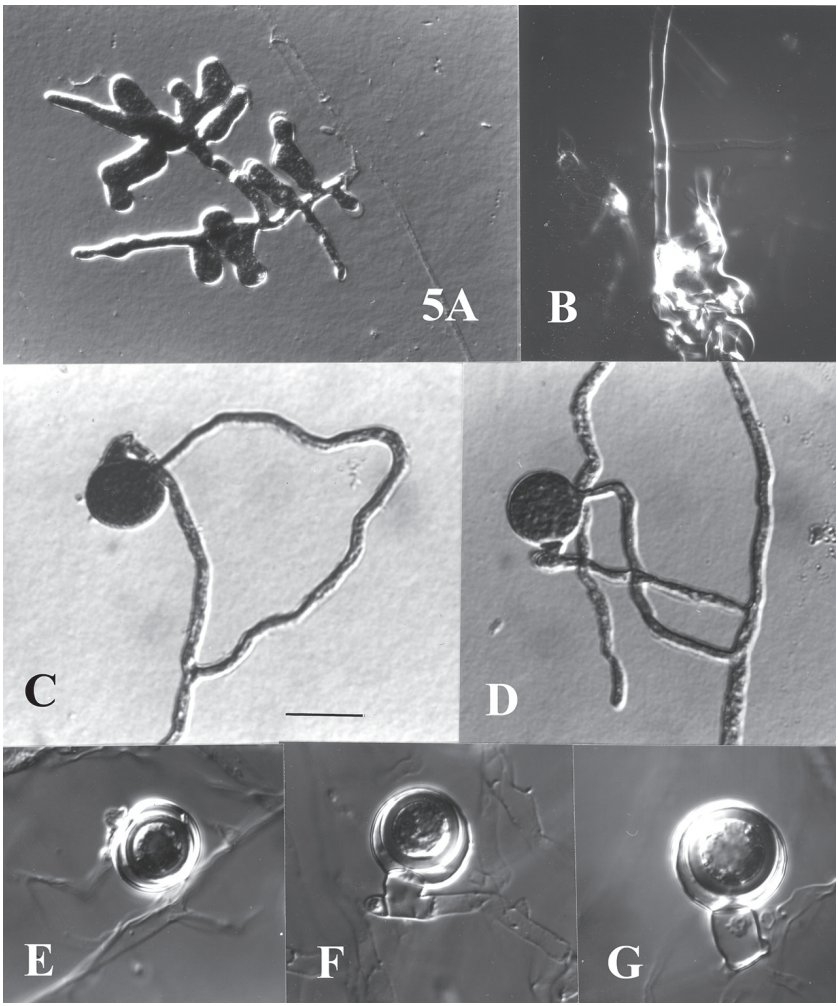

Fig. 5. *Pythium deliense.* **A, B.** lobulate sporangia, sporangium empty with long exit tube in B. **C, D.** curving of oogonial stalk toward the antheridium (terminal in C and intercalary in D); **E–G.** oogonia with aplerotic oospores and antheridia (terminal and diclinous in E but intercalary in F and G). Scale bar: 20 μm (all at the same magnification)

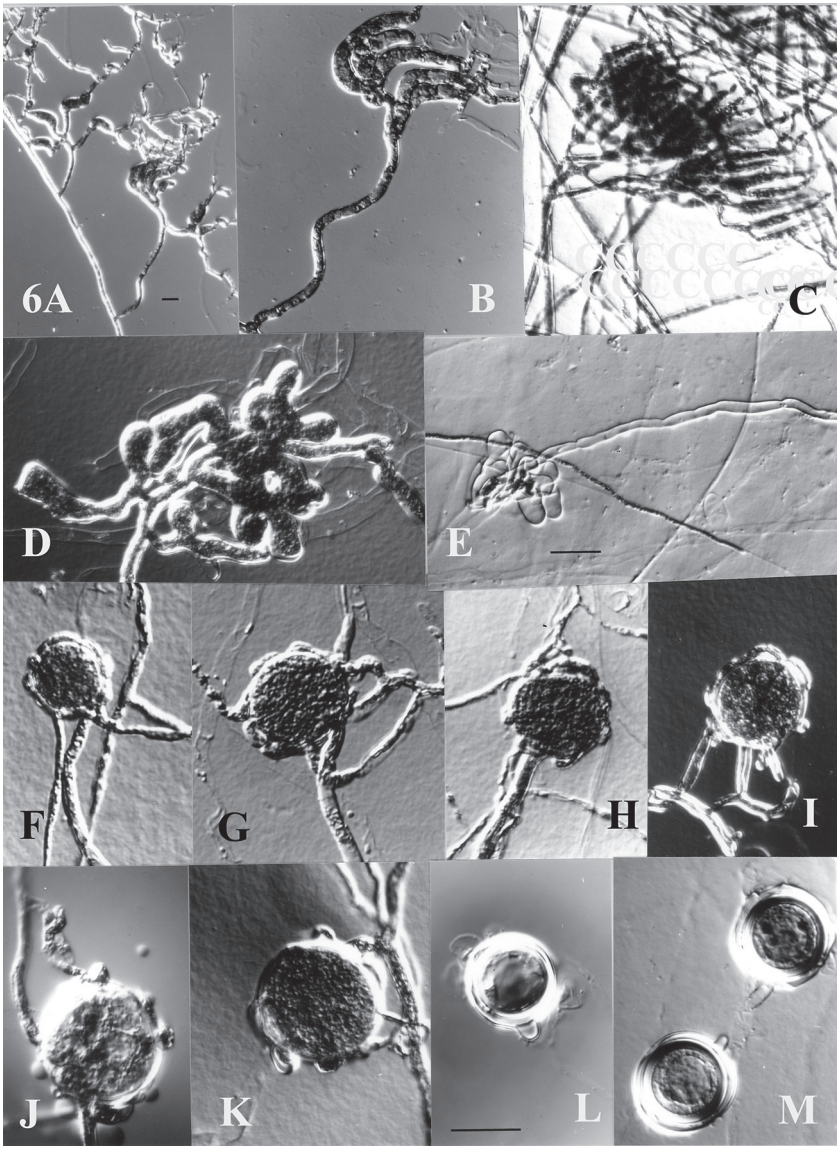

Fig. 6. *Pythium myriotylum*. **A–C.** appressoria, conspicuously broom-like in C; **D, E.** lobulate sporangia, empty sporangium with long exit tube in E; **F–K.** oogonia surrounded by club- or crook-shaped mostly diclinous antheridia; **L, M.** mature oogonia with mostly aplerotic oospores in M and an occasional plerotic oospore in L. Scale bar: 20 μm (all at the same magnification, except A)

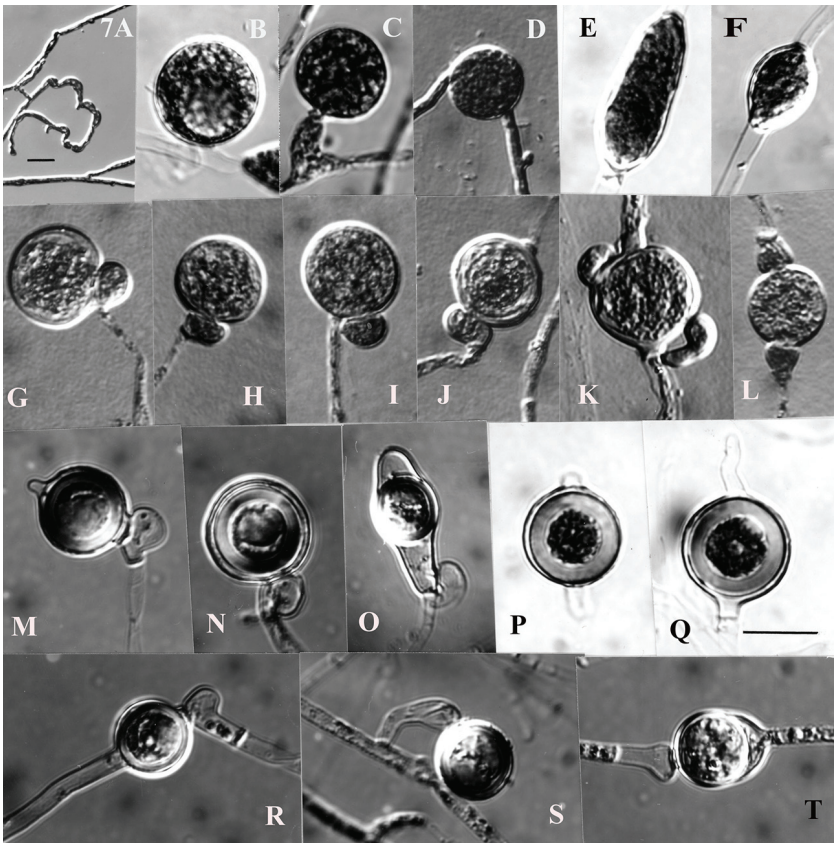

Fig. 7. *Pythium ultimum* var. *ultimum*. **A.** appressorium; **B–F.** hyphal swellings; **G–I.** terminal oogonia with monoclinous sessile antheridia attached to the base of oogonia; **J–L.** intercalary oogonia with monoclinous antheridia, sessile in J, on short stalk in K and hypogynous in L; **M–Q.** Mature terminal oogonia with thick-walled aplerotic oospore; **S, T.** intercalary oogonia with developing aplerotic oospores and monoclinous antheridia, sessile in R; on short stalk in S and hypogynous in T. Scale bar: 20 μm (all at the same magnification except A)

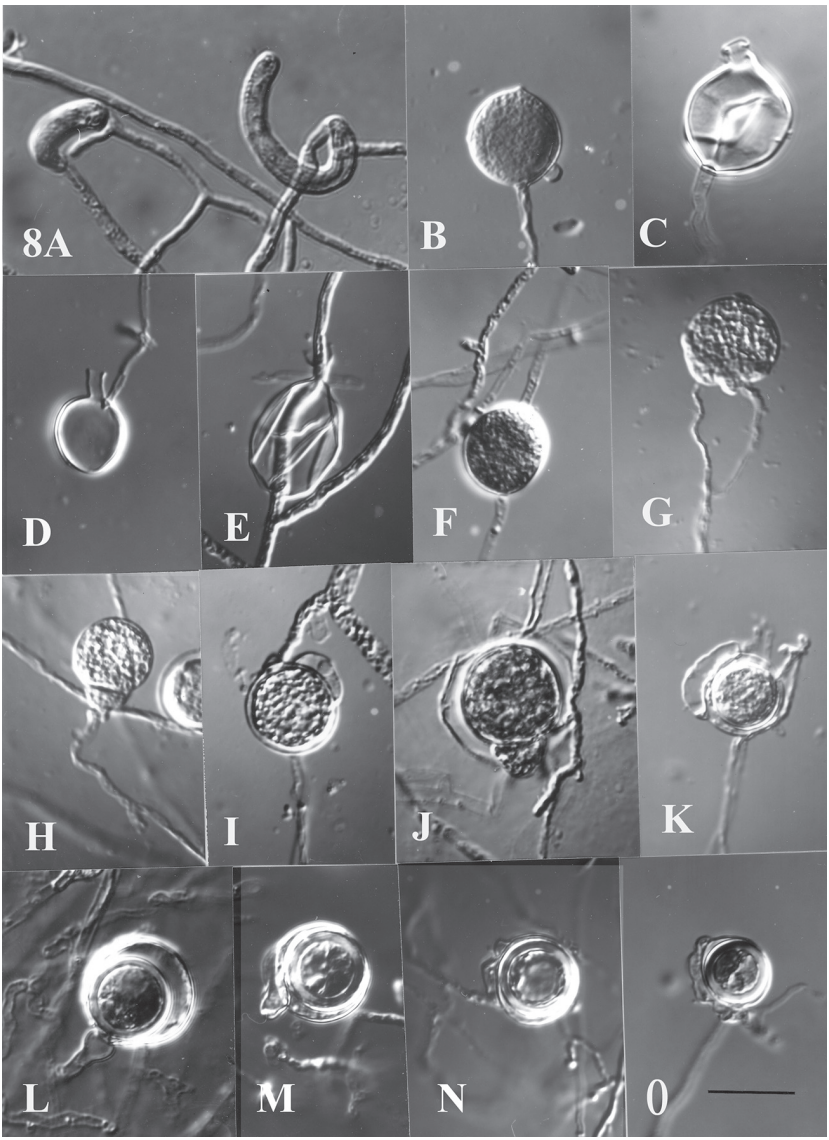

Fig. 8. *Pythium vexans*. **A.** appressorium; **B–D.** terminal sporangia , empty sporangia with short exit tubes in C and D; **E.** an empty sporangium proliferating internally; **F.** intercalary sporangium; **G–K.** developing oogonia with antheridia campanulate in H, I and J but irregularly elongated in G and K; **L–O.** mature oogonia with mostly aplerotic oospores in L and M but occasionally nearly plerotic oospores in N and O. Scale bar: 20 μm (all at the same magnification)

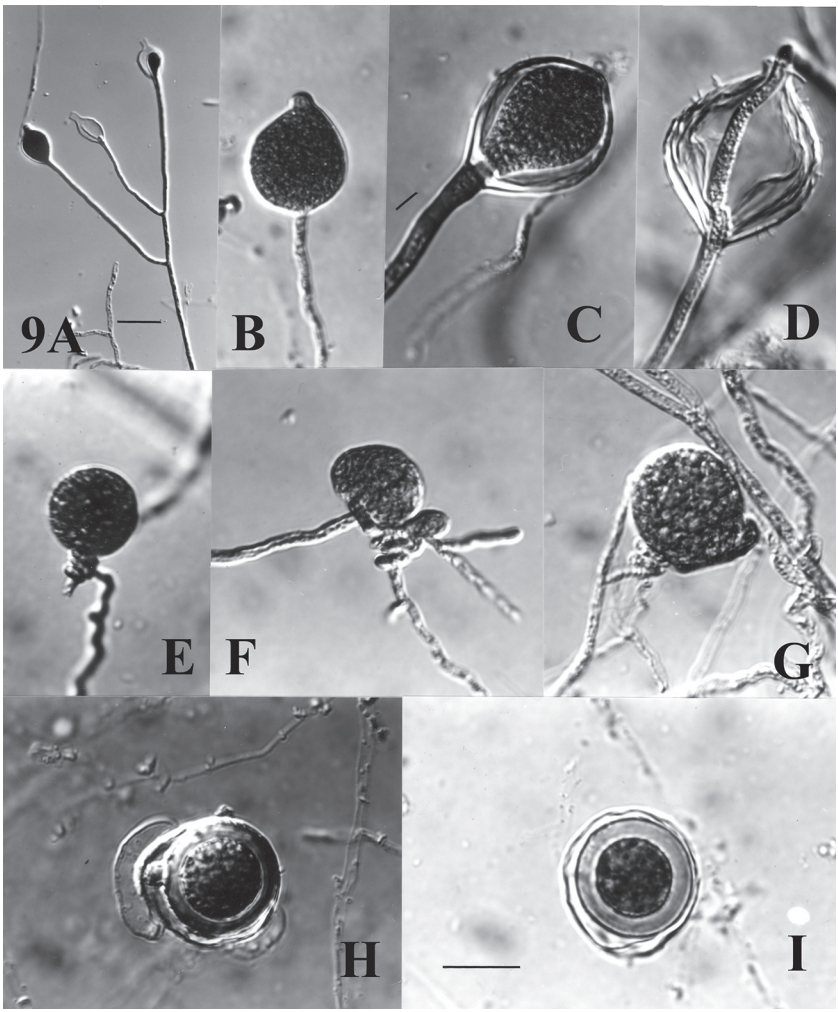

Fig. 9. *Pythium helicoides*. **A.** sporangiophore with cymose branching; **B–D.** terminal papillate sporangia showing internal proliferation in C and D; **E–G.** developing oogonia with antheridial stalk coiling around the oogonial stalk at the base of the oogonium; **H, I.** Mature oogonia with large elongated antheridia applied close to the oogonium in H with thick-walled aplerotic oospores Scale bar: 20 μm (all at the same magnification except A)

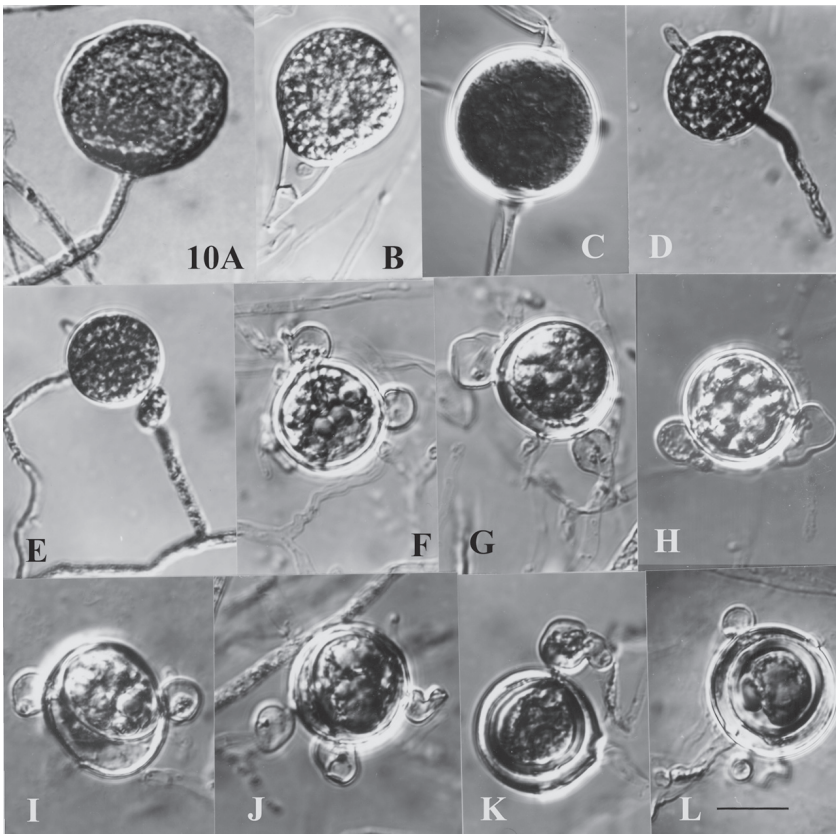

Fig. 10. *Pythium splendens*. **A–C.** hyphal swellings terminal in A and B but intercalary in C; **D.** hyphal swelling germinate by germ tube; **E.** developing terminal oogonium with diclinous antheridium; **F–L.** mature oogonia with aplerotic oospores and club- or crook-shaped antheridia. Scale bar: 20 μm (all at the same magnification)